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PATENT- OG VAREMÆRKESTYRELSEN

Title MICRODEVICE FOR INTEGRATED AIR SAMPLING, SAMPLE PREPARATION AND DNA AMPLIFICATION

Prior art:

US5,674,742

US6,586,253

US6,673,621

FIELD OF THE INVENTION

The invention relates to a microscaled device that can collect particles containing or consisting of biological material and extract genetic material and amplify the said genetic material for subsequent detection.

1. BACKGROUND OF THE INVENTION

Introduction

In order to facilitate rapid detection of airborne pathogens capable of causing either natural or deliberate epidemics it is important to collect particles that holds or consists of the said pathogens in order to facilitate rapid detection. Diseases that are spreading through air pose a serious health risk to man. According to the World Health Organization (WHO) as many as 1000 million people may become infected with Tuberculosis (18, 22, 27, 39, 43, 60, 69, 74, 85) between 2002 and 2020, of which approximately 150 million will get sick and 36 million people will die from the disease. The first new communicable airborne disease of the 21st century is Severe Acute Respiratory Syndrome (SARS), which has the potential of generating rapidly spreading infections (30, 36, 37, 54, 65). Parallels can be feared with the spread of the Plague ("the Black Death") with Europe's Middle Ages, when approximately 25 million people died in the period 1347 to 1352. The most deadly form of the Plague is by inhalation of infectious aerosols, where as little as 1-10 cells of *Yersinia pestis* are sufficient to cause disease. The aerosols can arise by respiration of infected humans or, more threateningly, from deliberate releases *Y. pestis* by bioterrorists (5, 14, 17, 31, 87, 92).

Biological warfare (BW) agents of critical concern are bacterial spores, such as *Bacillus anthracis* (anthrax) (15, 20, 27, 40, 52, 53, 82, 90-93), *Clostridium tetani* (tetanus), and *Clostridium botulinum* (botulism). Spores, produced by certain types of Gram-positive bacteria in response to starvation, are non-growing, heat-resistant, dehydrated, and resistant to extremes of temperature, pH, desiccation, radiation, and chemical agents. This stability makes them an attractive tool for use in BW weapons.

Other micro-organisms with the potential of being used to generate deliberate epidemics comprises micro-organisms causing e.g., Smallpox (4, 69, 89), ADDINEbola (6, 45, 70, 80), Encephalitis (8, 16, 38, 68, 79) and Q-fever (3, 10, 27, 41, 44). Also feared are the natural occurring recombination products of airborne influenza (23, 29, 57, 66, 69, 75, 88) with the deadly Avian flu (1, 7, 13, 24, 28, 33, 59, 62, 67, 83, 84). These recombined viruses have the potential of causing pandemics facilitated by air travel of as of yet unseen dimensions. This invention comprises the design of a device aimed to be simple in construction, nevertheless allowing advanced diagnostics to be carried out, with near instantaneous detection (within a few minutes) making ultimate use of the sample in the process. These points are of high importance for mobile detectors, as it is critical to detect bioaerosols containing disease causing organisms at the lowest possible concentration. Loss of genetic material from the samples results from extensive handling and complex laboratory procedures (19, 32, 86). It is therefore, when working with single particle preparations, highly advisable to process the specimen directly in the container, wherein the sample preparation has taken place. Subsequently, DNA amplification preferably should be performed in the same compartment, preventing excessive handling of the extracted genetic material.

A related issue is to identify methods that are all compatible, meaning that they do not interfere with each other. Ideally, the techniques should be able to be combined into a miniaturized design that allows for the capture, sample preparation, and DNA amplification of a single bioparticle in an industrial suitable form. The presently described invention is an industrial solution to this. Said invention will be applicable in many different fields in diagnostics and molecular biology research, while having the potential of dramatically transforming the monitoring and control of infectious diseases.

The capture of bioparticles in a microstructure

As outlined by Thomsen et al (patent application ####1) it is possible to capture particles containing or purely consisting of biological material in a microstructure by

applying an electrical field across the path in which the said particles are passing. Charges carried on the particles forces them to settle on either the anode or the cathode over a given distance as function of their initial charge, velocity, start position and the applied electrical field. In order to find the most effective design for capturing bioparticles by means of an electrostatic field, Thomsen et al. compared and tested different biophysical models and found that a long channel with two opposing electrodes was superior in order to facilitate the highest capture rate, whereas point capture could be facilitated by other structures.

Simplified model for capturing bioparticles

Thomsen et al optimized their sampling of bioparticles by adjusting the parameters of a truly multi-dimensional situation given by linear and non-linear parameters describing:

a) channel, electrode and capture area geometry
b) spore properties as size, density, initial charge
c) air flow rate
d) applied potential and direction of field lines.

In order to obtain an estimate for the idealized capture efficiency, firstly a simplified theoretical analysis was performed assuming uniform velocity of all particles, in viscid fluid, uniform field and field charging of spores. In this way an expression was obtained for the idealized capture length given by:

$$L_c = \sqrt{\frac{\rho_p d_p H \xi}{18 \varepsilon W^2 V^2}}$$

Equation 1

Where:

W	=	channel width
ε	=	electrical permittivity of air
ρ_p	=	spore material density
d_p	=	spore size
ξ	=	air flow rate

Following the modeling Thomsen et al. showed that the theory was applicable and that bioparticles could be caught in a microstructure with a capture rate above 80%. Since the capture structure can be made of two parallel mounted thin films of a conductive material it seems ideal for integration with the other technologies relaying on thin film mediated biophysical experimentation. The thin films for capturing of bioparticles could

have dual use and could be utilized in both high frequency electrical field sample preparation and as heat elements in the thermo-cycling to perform PCR.

The sample preparation of spores from Gram positive bacteria

Thomsen et al. (patent application ####2) showed that it is possible to extract DNA from from endospores of gram positive bacteria by means of high frequency alternating electrical fields. It was demonstrated that the sample had to be located between two parallel sheets of thin film in order to facilitate release of DNA suitable for amplification by PCR. According to the experiments a 5 second period of 10 V with a oscillation at 100 KHz was enough to release target DNA from a solution containing endospores and with a concomitant PCR reaction. The process was not detrimental to the PCR process and is therefore ideal to implement in an integrated device.

Thermo-control of a microstructure

When designing a device to perform PCR there are two factors of prime concern; speed of heat transfer and PCR-compatibility. Various surfaces can negatively influence the PCR reaction by the adsorption of materials or the inhibition of the enzymes. Fortunately, prior art exists on the treatment of surfaces for the materials that are likely to be used. Given that a high speed PCR reaction is required, considerable attention has to be given to the transfer of heat into and from the PCR mixture. A successful design lies in the careful trade-off between the thermal and biological performance, to optimize the device for the particular application scenario.

The main aim of this invention is to give precedence to speed and sensitivity of the PCR process. In any PCR process the means of heating and cooling of the sample is important for the final result. As high speed is a key consideration, heat transfer to and from the sample is the primary concern.

The PCR Cycle

It is important to have an outline of the PCR cycle protocol that is considered. High speed PCR is generally regarded to have cycle times below 30 s. Very fast thermo-cycling capability has been demonstrated by performing 30 cycles within 2.5 min (5 s per cycle) without DNA amplification (12). However, a fast cycling PCR with low yields has also been reported (34).

The model of said invention analyzes a PCR-cycle with a denaturing step at 94 °C for 0.5 s, followed by an annealing step at 50 °C for 1 s, and finally, an extension step at 72 °C for 3.5 s. There is clearly some tolerance on these temperatures, for example denaturing at 94 °C ± 3 °C, annealing at 50 °C ± 0.5 °C , extension at 72 °C ± 1 °C . In general, the more rapid the transitions, the faster the times and the more accurately the temperatures are achieved, the more specific the amplification will be. It is generally held that the denaturing and annealing processes occur very rapidly once their target temperatures have been attained. The very short dwell times at the target temperatures imply that the transitions between temperatures will also be short. In the example above the total of the dwell times equal the 5 s/cycle allowed if 30 cycles are to be achieved in 2.5 min. Even if a somewhat relaxed target is acceptable, none of the dwell times will be long. However, the temperature transitions should be short compared to the dwell times, suggesting transition times of well under 0.5 s.

Melting of double stranded DNA into two complementary single strands is a critical step for PCR to occur. The melting time can be shortened by increasing the temperature from 94 °C to 96 °C. In standard PCR reactions, rising the melting temperature leads to increasing loss of polymerase activity. However, in fast cycling PCR the dwell time at the high melting temperature is very short and therefore the enzyme is not subject to any considerable inactivation even at 96°C.

Basics of Heat Transfer

Essentially, the discipline of heat transfer is concerned with only two things: temperature, and the flow of heat. Temperature represents the amount of thermal energy available, whereas heat flow represents the movement of thermal energy.

On a microscopic scale, thermal energy is related to the kinetic energy of molecules. The higher the temperature of a material, the greater will be the thermal agitation of its constituent molecules. Regions that contain greater molecular kinetic energy (i.e. higher temperature) will transfer this energy to regions with less kinetic energy (lower temperature).

Several material properties can modulate the transfer of heat between two regions of differing temperatures. Examples include thermal conductivities, specific heats, material densities, fluid velocities and viscosities, surface emissivities, and more.

Heat Transfer Mechanisms

Heat transfer mechanisms can be grouped into 3 broad categories:

Conduction: Regions with greater molecular kinetic energy will transfer their thermal energy to regions with less molecular kinetic energy through direct molecular collisions, a process known as conduction.

Convection: When heat is conducted into a static fluid, it leads to a local volumetric expansion. As a result of gravity-induced pressure gradients, the expanded fluid parcel becomes buoyant and displaces, thereby transporting heat by fluid motion (i.e. convection) in addition to conduction. Such heat-induced fluid motion in initially static fluids is known as *free convection*.

For cases where the fluid is already in motion, heat conducted into the fluid will be transported away chiefly by fluid convection. These cases, known as *forced convection* or *advection*, require a pressure gradient to drive the fluid motion, as opposed to a gravity gradient to induce motion through buoyancy.

Radiation: All materials radiate thermal energy in amounts determined by their temperature, where the energy is carried by photons of light in the infrared and visible portions of the electromagnetic spectrum. When temperatures are uniform, the

irradiative flux between objects is in equilibrium and no net thermal energy is exchanged. The balance is upset when temperatures are not uniform, and thermal energy is transported from surfaces of higher to surfaces of lower temperature.

A note on geometry

This invention considers rather small volumes of fluid, in the range from 100 nl to 500 µl, typically 10 µl. Aimed at rapid thermal cycling, obviously the volume of the containing structure should not add excessively to the liquid volume. The liquid may be arranged having similar dimensions in all axes, e.g. a cube or sphere, to have similar dimensions in 2 axes with the third axis shorter, e.g. a disc or planar form, or similar dimensions in 2 axes with the third axis longer, e.g. an elongate cylinder or capillary form. The cubic or spherical forms are discounted since at the highest volumes in the range it would be rather limiting in terms of thermal performance, whereas as the volume decreased it would become inconveniently small to handle. This invention assumes a planar form unless indicated otherwise.

Cooling and heating—some ground rules

For both heating and cooling, heat must transfer between the liquid sample in the chamber and "the outside world". In particular, heat must transfer through the bulk of fluid sample, through the walls of the chamber and to (from) the outside of walls to the external source (sink). As outlined previously, four modes of heat transfer should be considered in this application: radiation, conduction, convection, and advection. The amount of heat to be transferred can be estimated as the amount of heat required to heat a volume of water equivalent to that of the fluid sample between the minimum and maximum temperatures of the PCR cycle. This temperature difference is approximately 50 K, and so the heat to be transferred per cycle is approximately 2 Joules for a 10 µl sample volume.

Radiation

It is helpful to consider heat transfer by radiation first. All surfaces emit thermal radiation. However, at any given temperature and wavelength, there is a maximum amount of radiation that any surface can emit. A surface emitting this maximum amount of radiation, it is known as a blackbody. There are well known equations, such as Plancks Law that can be used to calculate the amount of radiation emitted as a function of wavelength and temperature. Most surfaces are not blackbody emitters, and emit a fraction of the amount of thermal radiation that a blackbody would. This fraction is known as emissivity. If a surface emits ½ as much radiation at a given wavelength and temperature as a blackbody, it is said to have an emissivity of 0.5. If it emits 1/10 as much as a blackbody, it has an emissivity of 0.1 and so on. Obviously, a blackbody has an emissivity of 1.0 at all temperatures and wavelengths.

The rate of heat transfer is:

$$A\sigma\varepsilon(T_1^4 - T_2^4)$$

where A is the area, in m^2, through which irradiative transfer occurs, σ is the Stefan–Boltzmann constant ($5.67 \cdot 10^{-8} \mathrm{Wm^{-2}K^{-4}}$), ε is the emissivity of the radiating surface, and T1 and T2 are the absolute temperatures (in Kelvin) of the source and sink of the radiation. From this, the maximum cooling achieved by radiation can be estimated. This will occur when the source temperature is at its highest, i.e. during DNA denaturing, and with as low a temperature as practical as the sink temperature—for example a sink at 4 °C. For this calculation an area of 1 cm^2 is assumed, and of course the heat transfer will scale linearly with the change in area. In this case the rate of heat transfer, assuming an unrealistically high emissivity of 1, is:

$$10^{-4} \cdot 5.67 \cdot 10^{-8}(371^4 - 277^4)\mathrm{W} \approx 70\mathrm{mW}$$

Since the total heat to be transferred is approximately 2 J for a 10 µL sample it is apparent that irradiative heat transfer alone will not allow for a rapid cycle time. Of course if we want to heat the sample there is opportunity to have a radiation source at

considerably higher temperature and thus obtain a much faster heat transfer. Assuming a sample temperature of 90°C (that is approaching the denaturing temperature) with a radiation (heater) source at 750 °C, that is a "red hot" heater, and then the heat transfer rate is:

$10^{-4} \cdot 5.67 \cdot 10^{-8}(1023^4 - 363^4)W \approx 6W$

This rate of heating is sufficient for the enablement of a rapid cycle, and could be further increased by adding or moving to a still hotter radiation source.

It can be seen that radiation under "realistic" conditions is able to transfer heat into the sample almost 100 x faster than it can get it back out. In these simple calculations the wavelength of the radiation is not considered. This is an over-simplification, particularly in the case of infra-red heating of water. Fortunately, over a significant part of the near infra-red spectrum, water is strongly absorbing rendering the conclusion valid. Said calculations illustrate that in said type of application, cooling the sample is a more fundamental problem than heating it.

Given that irradiative heat transfer is unable to cool a sample sufficiently fast, other means of heat transfer must be employed for cooling with every probability that the same means should be used for heating. As cooling is the more fundamental problem, means other than irradiative heat transfer for cooling should be examined.

Conduction

Cooling may be provided by a conductive link to a heat sink. Commonly, this is employed in conventional PCR systems by placing the sample container onto, or into, a cooled block.

Conduction plays a fundamental role in determining the thermal properties of any system, as this is the mechanism by which heat will flow through both the solid parts of the structure and within the small sample volume.

The rate of heat transfer by conduction is given by:

$$\dot{Q} = \frac{KA\Delta T}{d}$$

wherein $\dot{Q}$ is the rate of heat flow, K is the thermal conductivity of the conducting medium, A is the area through which conduction takes place, d is the thickness of the conducting material, and ΔT is the temperature difference which drives the conduction. This thermal conduction equation can be seen to be analogous to electrical conduction, which is governed by Ohm's law commonly given as $V = IR$, or in analogy to the thermal conduction equation above $I = \frac{V}{R}$. From equation XX it is obvious that thermal resistance should be considered. When heat is conducted through a series of materials the thermal resistance through the series is the sum of the thermal resistances through the individual elements. An important conclusion from this is that the thermal resistance of the least conductive material may dominate the overall resistance. In practice this means that minimizing the thickness of polymer layers and eliminating air gaps is far more important than the difference between metals and/or ceramics in the highly thermally conductive portions of a system.

In Table 1 typical thermal properties are given - the values for water may be used for the sample. From these values it is apparent that the sample (water) has the highest thermal capacity per unit volume, and that polymeric materials have a much lower thermal conductivity than metals.

Thermal capacity is entirely analogous to an electrical capacitance, with similar equations applying. From Table 1 it can be seen that polystyrene (a typical plastic) has a relatively high thermal resistance and further that water has a relatively high thermal capacity.

Recalling that in an electrical circuit a time constant for the circuit is the product of the resistance and the capacitance it can be surmised that transfer of heat through a thick-walled plastic vessel into a volume of water will have a long time constant. From this it is apparent that otherwise convenient vessels such as Eppendorf tubes have limited suitability for a rapid PCR device.

Table 1: Typical thermal properties of selected materials.

Material	Thermal conductivity ($Wm^{-1}K^{-1}$)	Heat Capacity ($Jcm^{-3}K^{-1}$)†
Water	0.65	4.2
Copper	397	3.45
Aluminum	239	2.4
Silicon	130	1.4
Alumina ceramic	20	
Glass	1.0	1.74
Heat sink paste	0.9	
Polystyrene	0.08	1.37
Air	0.0241	$2.4x10^{-5}$

Notes † Heat capacity is tabulated in units of Joules per milliliter per Kelvin, since a volumetric heat capacity is more directly useful in this case than the usual expression of Joules per kilogram per Kelvin.

Heating and cooling to equilibrium—the importance of thermal time constants

Provided that there is a suitable simple time constant for the system then the heated / cooled sample will approach its final temperature exponentially. Table 2 illustrates the response of such a system to an imposed temperature change. For convenience a temperature change over a range of 100 °C is tabulated, as the Table may also be used to illustrate the number of time constants that must elapse for a system to attain a given percentage of the imposed temperature change.

Table 2: Response of a system with a simple time constant to an imposed temperature change.

Time constants	Temperature change in sample (°C)
0.0	0.00
0.1	9.52
0.2	18.13
0.3	25.92
0.4	32.97
0.5	39.35
0.6	45.12
0.7	50.34
0.8	55.07
0.9	59.34
1.0	63.21
1.5	77.69
2.0	86.47
2.5	91.79
3.0	95.02
3.5	96.98
4.0	98.17
4.5	98.89
5.0	99.33
6.0	99.75
7.0	99.91

Considering a temperature change from the denaturing temperature (94°C) to the anneal temperature (50°C) and noting that the annealing temperature is to be achieved to within ± 0.5°C , Table shows how many time constants would have to elapse for this system to reach an adequate equilibrium. The system needs to have reached approximately 99% of the equilibrium temperature change, which corresponds to approximately 4.5 time constants. If the temperature transitions are to be considered as "fast" then reasonably the transition time should be short compared to the hold time at the target temperature. In the case of very fast PCR this requires that the transition time should be short compared to one second. A transient time of 250 ms might be considered adequately fast, suggesting sustained cooling rates of 160 °C/s for the widest transition (from denature - to annealing temperature). This cooling rate is in excess of those reported, for example Northrup et al. report heating rates of 10 °C/s and cooling of 2.5 °C (56); Lagally et al. achieved 20 °C/s (35); the Landers infrared heating device achieved heating rates of 60 °C/s and cooling of 20 °C/s, while Daniel has a silicon structure which achieved 74 °C/s (12).Unfortunately, as stated previously, it is more difficult to cool the sample than to heat it. Additionally, the widest temperature transition occurs when cooling.

When the data in Table 2 are presented as a graph, and shows the transition 94 °C to 50 °C, as in Figure 1, then the cooling of the sample can be seen. Figure 1 also shows the tolerance band at ±0.5 °C of the target temperature, of which the upper region is reached in 4 to approximately 4.5 time constants from the start of cooling. In Figure 2 the same cooling curve is shown, with a magnified view of the section approaching the target temperature. This clearly illustrates that the sample is close to the target temperature but not close enough to be within the tolerance band for an appreciable time. This emphasizes that any simple approach of moving a sample vessel from one temperature zone to another and allowing equilibrium at the new temperature will not have any effective high precision of temperature control unless the time constant for the system is very short. In effect, as accurate temperature control is essential for the

reduction of non-specific amplification, a system with very fast temperature transients is required whenever non-specific binding is to be minimized — regardless of the cycle time requirement. Thus, if the temperature transients are to be as fast as possible, the system must be forced to the required temperature and then controlled at it. For example, at the cooling phase, approaching the annealing point; the device should behave as though it is cooling to a far lower temperature than is required (for example as though cooling to room temperature). When the temperature reaches the intended temperature, or even undershoots it marginally, heating should be reapplied to reduce and then balance further heat efflux and thereby stabilize the temperature of the sample at the anneal point. From this consideration it is concluded that in the ideal device there will be a heater and temperature sensor in the sample envelope. The sample envelope being thermally linked to a heat sink maintained at a temperature lower than the annealing temperature, and the heater will be operated so that it always opposes the conductive heat leak from the sample to the exact degree required to maintain or change the temperature. In such an arrangement the heat source could be either a resistive heater or a radiation source. If a radiation source is used then careful consideration of location of the source and the wavelength is required. The wavelength selected will determine the rate of energy absorption in the sample, the sample envelope, and other components of the system.

Having ascertained that a very short time constant is needed, it is reasonable to consider how short a time constant is achievable realistically. In the case of a thick sample layer that is contacted to a heat source / sink through a plastic wall, it is rather easy to estimate the time constant. The thermal capacity is likely to be dominated by the sample, whereas the resistance is dominated by the plastic. In the example below the values for polystyrene are used, but the general principle is equally valid for other plastics.

Considering a simple case of a 1 cm^2 square plastic envelope with a wall thickness of 100 µm, containing 10 µl of sample, i.e., a sample thickness of 100 µm. Assuming this

sample envelope to be placed with one major face onto a heated block and that the other faces are in contact with free air. The volume of plastic material in the envelope (ignoring the edges) is 0.02 ml, which for typical plastic material gives a heat capacity of 0.027 J/K. The sample (10 µl) has a heat capacity of 0.042 J/K. So in this example 60% of the heat capacity remains in the sample. The thermal resistance through the plastic face is given by

$$R_{\text{Plastic}} = \frac{t_{\text{Plastic}}}{\kappa_{\text{Plastic}} \cdot A_{\text{Plastic}}}$$

wherein t, k and A are respectively the area, the thickness and the thermal conductivity of the material through which the heat is transferred. The subscript "plastic" relates in this case to the plastic material.

In this example we have:

$$R_{\text{Plastic}} = \frac{10^{-4}}{0.08 \cdot 0.01 \cdot 0.01} = 12.5\text{KW}^{-1}$$

This is the thermal resistance through one face of the plastic envelope. The thermal resistance through the entire thickness of the sample is:

$$R_{\text{Sample}} = \frac{1.0 \cdot 10^{-4}}{0.65 \cdot 0.01 \cdot 0.01} = 1.54\text{KW}^{-1}$$

This is an over-estimate as not all of the heat flows through the entire sample. Almost 90% of the thermal resistance is in the plastic envelope. This simple example configuration can be expected to have a time constant approximated by:

$$R_{\text{Plastic}} \cdot C_{\text{Sample}} = 12.5 \cdot 0.042 \approx 0.53\text{s}$$

This relatively simple system has a time constant equal to the dwell time that is likely to be required. If the system is to equilibrate several time constants are required to elapse, and the transient time should be significantly shorter than the dwell times. Hence this system has a time constant several times longer than can be employed.

The system above is highly simplified, especially as it assumes that the heat source / sink acts as an infinite source / sink, i.e., the temperature of the source / sink does not change as a result of the PCR envelope being placed on it. However, for a system of this type it is a reasonable approximation—provided that the heater is sufficiently large and made of normal thermally conductive metal or ceramic. As very fast PCR is considered, this should be modeled if the preferred cycling scheme makes such modeling appropriate.

The need for several time constants for the imposed temperature change to take effect is a consequence of allowing the system to equilibrate against a fixed temperature source. Nevertheless, more rapid temperature changes are possible, but to achieve this more sophisticated control is required, which poses a new set of challenges.

In the simple system previously described, a simple all-plastic envelope of reasonably robust construction was used; essentially an extrapolation of the thin-walled PCR tube to a reasonable limiting thickness.

Under the above conditions, the requirements of fast PCR are not compatible with an all-plastic sample envelope. Table 1 shows thermal properties of some candidate substitute materials. Clearly copper has the most attractive thermal conductivity, but it is rather toxic to the reagents. Aluminum and silicon have smaller thermal conductivity, but are still remarkably better than plastic. Both Aluminum and Silicon have the advantage that mechanically stable, chemically inert oxides that can be grown or deposited with standard processes, and both (these oxides) are more immediately compatible with many of the thin layer polymers used in micro-engineering than copper. Alumina is a readily available, low-cost ceramic with good thermal properties, but not as good as those of aluminum or silicon. In comparison with silicon it is rather a rough surface, so that the effective surface area is much larger than the apparent area. This may be a significant factor if there are biocompatibility issues, or simply in that trace material may become embedded in open surface pores. Any of copper, aluminum, silicon or alumina

has an adequate combination of thermal conductivity and mechanical strength so that an envelope made of one of these materials in place of plastic could have an acceptable thermal time constant for conduction to allow rapid PCR. If these materials are used for the sample envelope then the thermal resistance of the sample is larger than that through the envelope. In the simple model given previously the time constant would drop approximately tenfold, reaching an acceptable value. The thermal resistance of these materials is so low that a relatively robust piece of material can be used subsequently increasing the thermal capacity of the sample envelope, which consequently, becomes larger than that of the sample. However, with this increased complexity a more complex thermal calculation is required to determine the response times.

For PCR to take place the surface has to be biocompatible. Neither native surfaces of copper, aluminum, or silicon is adequately biocompatible, so some kind of surface coating has to be applied. This coating has to be very thin in order to prevent the thermal properties of the substrate from being affected by a thin, but very thermally resistant, biocompatible coating and subsequently resulting in extended cycling times. Wilding et al., give a useful review of treatments for silicon and glass surfaces, with many other references containing specific "recipes" used for the particular device discussed (78). In particular, many different silane treatments have been used on glass and silicon oxide surfaces.

As can be seen in Table 1, the thermal characteristics of glass are better than those of plastic, but its thermal conductivity is still less than 1 % that of the thermally better materials. To be compatible with rapid PCR, glass would be rather too thin to be mechanically robust over large areas. However, it is possible to use glass as a thin coating, for example on a silicon substrate, the "glass" being conveniently produced by a wet thermal oxidation of the silicon surface—resulting in a film of typically 400 nm thickness.

Using a glass capillary design, the cylindrical symmetry gives mechanical strength and the overall small extent in two dimensions permits flexibility. To minimize breakage glass capillaries can be coated with a thin film of polyimide. However, while reducing damage due to handling even a thin layer of this polymer can provide a high thermal resistance in the context of rapid PCR.

It is of outmost importance that the side in contact with the heating / cooling system should make a physical / areal contact as complete as possible. This means that the surface should be both clean and flat. Table 1 shows that the thermal conductivity of air is some 3 x smaller than that of a typical plastic, but some 5000 x smaller than that of silicon suggesting that any air gaps are critical to the thermal cycling. Fortunately, the very high thermal conductivity of copper, silicon or aluminum is sufficient for heat to efficiently spread laterally in the material providing conduction paths around small air pockets (resulting from slight imperfections in contact between the outer face of the substrate and the heating (cooling) block).

It is generally preferable for heat exchange to take place through one major face of any planar sample-containing envelope, with the other face being essentially adiabatic. This confines the sample thickness to half of the thickness that might be used if heating and cooling took place from both faces simultaneously. However, this is a reasonable compromise between the surface-area to volume ratio and complexity of the device. In another embodiment an arrangement where heating takes place through one major face and cooling through the other could be considered. However, this is inappropriate to rapid thermal cycling systems and only attractive from the implementation point of view. In order to achieve rapid cooling the thermal resistance through the walls of the sample-containing envelope and onto the ultimate heat sink should be kept small, ideally to no more than the thermal resistance through the thickness of the sample. In these circumstances heating from one side of the sample while cooling through the other can only result in unacceptably large thermal gradients across the sample.

Convection and forced convection

As well as thermal radiation and conduction, convection should also be considered. For rapid PCR devices natural convection is of minor consequence, as only laminar flow occurs and the rate of heat transfer is too low to be of significance.

Forced convection (advection) is, however, able to transfer heat at adequate rates to support rapid PCR. The calculations for forced convection are more complex than those for conduction. Expressions provided in Perry are used herein (Perry 50th Ed.).

In forced convection the heat transfer takes the form

$$q = h \cdot A \cdot \Delta\theta$$

where h is a heat transfer coefficient and is generally a complex function of the Reynolds and Prandtl numbers, A is an area relating to the system geometry, and $\Delta\theta$ is the temperature difference between the surface of the solid and the bulk of the fluid. Convection only transfers heat to (from) the surface of the solid wall of the container, so that a three stage process has to be considered:

1. transfer of heat from the bulk of the convective fluid to the external wall of the sample envelope
2. transfer by conduction through the solid wall of the sample envelope
3. conduction through the bulk of the liquid sample

The mathematics is most tractable if it is assumed that the rate of heat transfer into the wall of the envelope is the same at its interior and exterior surfaces, i.e. that the liquid heats the interior of the wall at the same rate that the convecting fluid cools it.

Accepting the simplification that heat flow into and out of the envelope wall is always balanced the rate of heat transfer by convection is equated to the rate of heat transfer from the sample

$$-C_{\text{sample}} \frac{\mathrm{d}\Delta\theta}{\mathrm{d}t}$$

In the latter case, mentioned above, the negative sign indicates that the heat flow balances the convective flow (net flow into wall is zero),

C_{sample}

is the heat capacity of the sample, and

$\frac{d\Delta\theta}{dt}$

is the time rate of change of the temperature difference between the sample and the convecting fluid.

For convenience the various factors which relate to geometry, flow rate and material properties are contained in a single factor K, to give a final expression

$$t = K^{-1} \cdot \ln\left(\frac{\Delta\theta_1}{\Delta\theta_2}\right)$$

Calculations for some representative cases are tabulated in Table 3. These calculations show that for convective heat transfer, cylindrical geometry is strongly preferred over planar.

Reducing the sample thickness, e.g. to 25 μm, would bring the transition times towards the acceptable range. Conversely, as the sample thickness decreases the effect of the thermal capacity and conduction of the walls becomes increasingly important and it is unlikely that a simple scaling of time with thickness would hold. It is, therefore, unlikely that adequate cooling rate could be achieved with air for a planar structure. Also in greatly reducing the sample thickness any surface effects, which could interfere with the PCR reaction, may become significant. PCR in thin layers has not been reported. The greater heat capacity of water suggests that it may be able to exchange heat at an adequate rate—provided that some means were established to switch the water jets with sufficient speed.

Table 3: Representative times for temperature changes imposed by convective flow

Geometry	**Sample thickness / diameter (mm)**	**Convecting matter**	**Flow rate (ms^{-1})**	**Time t for $\Delta\theta$ to change from 50°C to 1°C (s)**
Cylindrical	1	Air	150	3.6
Cylindrical	1	Air	50	6.0
Cylindrical	0.5	Air	150	1.2
Cylindrical	0.5	Air	50	2.1
Planar	0.5	Air	150	14.3
Planar	0.5	Air	50	34.4
Planar	0.5	Water	1	1.5

Conclusions from a simple thermal consideration

Irradiative transfer is far too time-consuming to be used for cooling the sample, but might be used for heating.

Thermal conduction can be suitably rapid for high-speed PCR provided that care is taken to minimize low thermal conductivity materials in the construction. In particular air gaps should be avoided and plastic thickness should be kept to the minimum required for biochemical surface compatibility. Glass is preferably to plastic, and thin layers are necessary for good response times.

Convection can be used to transfer heat to (from) the outer walls of the sample container. In this case a cylindrical geometry is far preferred over a planar geometry.

Sample thickness must be low otherwise the internal resistance of the sample will prevent rapid thermal transients.

Temperature measurement and control

The simplest means of controlling the temperature is to place the sample-containing envelope in thermal contact with a fixed temperature heat source (sink) and wait for

thermal equilibrium. The sample temperature then matches that of the heat source. This is the approach taken by traditional thermocyclers, in which a PCR-tube is located in a well in a fixed temperature block and allowed to equilibrate. As discussed previously there are two distinct drawbacks to this approach. The first is that adequate equilibration can be a relatively slow process; the second is that the rate of heating (cooling) exponentially approaches the equilibrium so that the effective precision of temperature control is potentially poor, leading directly to non-specific binding.

If the equilibrium approach is not taken then some means of monitoring the sample temperature must be found. From the point of view of cost this is better located in the instrument controlling the PCR process, whereas from the point of view of accuracy it should be located within the sample envelope. When the sample is large it is possible to embed a small wire thermocouple in the sample volume. However, when rapid PCR is required the sample is generally too small to allow a wire thermocouple to be used. Therefore, in rapid PCR devices the embedded temperature sensors are usually thin film sensors: typically metal film resistors, the resistance of which varies predictably with temperature or thin film thermocouples. Platinum is a common choice for such embedded sensors since the film thickness can be chosen so that both resistive heating elements and temperature sensors can be formed in a single lithographic step in the same deposited film. Platinum resistance thermometers find wide application in many industrial areas and are thus well understood. Nonetheless, metals or combinations of metals can be used. For example, in a system designed to simulate high power consumption electronic components and to test advanced packaging methods for them a combination of a thin film nichrome (NiCr) heater with aluminum temperature sensors has been used(11, 81). Similar direct heating through silicon, in the context of PCR, have been reported by Burns (Burns et al., 1996) and by Daniel (12).

Given that the transition times are expected to be rather short (< 1s) the control is critical. Heating options for the device could be thin film heaters (ideally within the

sample envelope), Peltier devices, infra-red sources (preferably including diodes for fast modulation), or convection systems - perhaps employing a blended mix of a hotter and a cooler stream, or a porous element heater.

In order to get the fastest possible transients the best method is to use an excess of cooling applied to the envelope with a heating means sufficient to overcome the cooling to the extent required at any given time. To minimize temperature gradients in the sample, the heating means should be located either between the cooling means and the sample or it should permeate the sample, for example as infrared radiation at a weakly absorbing wavelength. As discussed by Landers et al. radiation can have the advantage of passing through the walls of the sample envelope with minimal loss, and thus, uniquely, heats the sample without heating the structure (e.g. Landers et al., 1998).

For optimal performance of the device, dwell times in the order of 0.5 s is preferred. Therefore, the transients should be less than 0.5 s in duration, implicating that the control system should be able to sense and respond in much less than 0.5 s, preferably in a few milliseconds. An analogue signal conditioning board feeding to a PCI-bus hosted data acquisition module will easily be fast enough. This leaves a consideration of "latency"; since modern computer operating systems multitask, care should be taken to ensure that the control task has sufficient priority that it is not neglected while the system performs other tasks.

Static or moving sample

To achieve the temperature cycling either the sample can be moved between heat sources /sinks or the heat can be moved to the sample. The conventional heated block thermocycler exemplifies the moving sample approach, in that the entire sample vessel is moved from one block to another. Systems such as that reported by Moore et al. take the opposite approach to the extreme (12). A sample container formed in a silicon substrate always directly connects to both heat sources (resistance heaters) and a cooling system (conduction to the bulk wafer) and it is the (electrically determined)

balance between these two that establishes the sample temperature. An intermediate position is taken by devices, such as those reported by Schneegass (76) and Kopp (34). In these "flow-through PCR" devices a microfluidic channel meanders over a series of controlled temperature zones. As the fluid in the channel passes through the device its temperature changes to match the zone through which it is passing. A different solution is taken by the LightCycler™ by Roche, which uses a rapidly flowing air stream to control the temperature of a sample contained in a glass capillary.

When very rapid systems are considered it is preferable not to have to move the sample envelope between temperature stages. Furthermore, having established that the temperature transients should complete in less than 0.5 s it is clear that to move the sample sufficiently quickly is not trivial.

Internal or external heating and cooling

Internal heating and cooling has great appeal from the point of view of rapid thermal cycling. By minimizing material between the heater and the sample, the time constant is minimized and control is improved. Simple resistors may be used in the base of a sample well, or attached to a thermally highly-conducting part of the envelope to introduce active heating within, or very close to, the sample envelope. These resistors are usually of the thin film metal kind (12), but thick film systems have also been used in a rather sophisticated flow-through system by e.g. Motorola.

To integrate active cooling within the sample envelope an external Peltier element can be used. Its temperature can be modulated reasonably rapidly with the rate depending on the thermal capacity of the element and the efficiency with which it can be operated. It is noted that Peltier devices are only a means of moving heat against a temperature gradient, at the expense of dissipation of electrical energy within the devices. Since the temperature of the ultimate heat source/sink for the Peltier device is not changing during

the thermal cycling, the Peltier element will not be able to operate at best efficiency. Typically, this means that the electrical energy dissipated in the Peltier element will exceed the heat transferred by it. This may mean that it is impossible to modulate the temperature of the Peltier device fast enough to achieve sufficiently rapid cooling using this type of device alone. If Peltier devices are used they are perhaps better used as a fixed (low) temperature sink with a separate heater determining the sample temperature. Of course, since the Peltier device still requires some external heat sink it is debatable whether their use adds value.

It seems probable that a great deal of the non-specific binding in the PCR reaction results from insufficiently fast thermal transients, most likely at the approaches to the annealing stage. Unfortunately, the transition to the annealing stage is a cooling transition. The pragmatic approach is an external cooling system with its rate of cooling maximized, especially close to the target temperature, by making the cooling system over-cool the sample and then introducing a balancing heat to the sample to control the sample temperature above that of the cooler. This approach is compatible both with thin film heaters integrated into the sample envelope and with external infra-red heating.

BRIEF SUMMARY OF INVENTION

The device is aimed to monitor airborne infectious diseases and is optimized for monitoring of low aerosol concentrations, with direct implications for surveillance of epidemics. The device consists of three integrated technologies hosted in the same container (volume ranging from 10 nl to 10 ml) allowing sampling of bioaerosols, sample preparation for extraction of genetic material with subsequent amplification of DNA or cDNA. The container is supplied by a common input and output for air and liquid. Initially, air is passed through the container in a stream originating by either overpressure at the input or vacuum at the output. The container is equipped with electrodes facilitating electrostatic precipitation. The electrodes are typically formed as two opposing sheets between which the air is passed. However, other configurations, e.g. a single sheet electrode combined with a single or a set of point electrodes can be used to direct the sampled particles to a given spot in the container. The electrical field between the electrodes measured at the shortest distance is below 1000 V/mm in order to prevent electrical sparking. Bioparticles carrying a natural charge are caught in the electrostatic field and their travel through the container is discontinued and they are directed against the electrodes where they precipitate. The capture mechanism is effective and readily a capture efficiency of 80% or more is achieved. The time for the electrostatic precipitation is easily set by external equipment controlling the voltage on the surface electrodes. The precipitation stops when the voltage is turned off. Once a desired volume of air has been processed through the container, the pumping of air can be stopped and the container can be filled with a liquid containing the reagents for DNA / cDNA amplification.

The collected and concentrated bioparticles can now be exposed for the sample preparation. We have demonstrated that endospores of Gram-positive bacteria, which are known to be extremely resistant to mechanical, chemical, and heat degradation, releases chromosomal and plasmid DNA when exposed to an oscillating electrical field across the container. The DNA release occurs within a few seconds if the frequency is

above 10 kHz and a maximum release is achieved around 100 kHz. The effect of the oscillating field is the destruction of the integrity of the spores by either direct membrane breakdown, pore forming in the endospore wall, or sudden osmotic swelling due to biochemical degradation of the spore with concomitant activation of otherwise buffered divalent cations as Mg^{2+} or Ca^{2+}. The wall of the endospore of Gram-positive bacteria is the most stable enclosing structure found in bacteria, viruses, or fungi, protecting the organism and allowing it to reside dormant under extreme harsh conditions for decades (2, 9, 21, 25, 26, 42, 46, 55, 58, 61, 63, 64, 71, 77). The sample preparation technology utilizing an oscillating electrical field is therefore effective in releasing genetic material from bacterial cells and spores. The oscillating field can be induced by the same electrodes used for electrostatic precipitation.

Following the initial electrical sample preparation the sample is exposed to heat and cool cycles by rapidly heating the sample to a denaturing temperature and subsequently cooling it. The temperature cycling mediates further breakdown of cells, spores and especially viruses which are unaffected by the oscillating electrical field releases genetic material. Viruses are generally genetic material embedded in protein structures, which quickly denature at higher temperatures. The rapid temperature oscillations (heating rates > 40°C /second and cooling rates > 15°C /second) are enabled by designing the container for optimal heat exchange. The thermal design is based upon the relation between the total thermal time constants of the materials compared to the thermal time constant of the water in the container. The overall heat capacity of the surrounding materials of the container and the coupling of these materials to each other and to an externally mounted heat sink are important factors in enabling the rapid temperature oscillations. Heat is supplied through a conductive thin film of e.g. Au or Pt in both the top and bottom of the container and it is important that the liquid forms a flat sheet in between the heaters in order to achieve a rapid heating and cooling.

The thin film is heated by passing current through the conductive material. The temperature is controlled by monitoring a four wired Wheatstone bridge thermo-sensor arrangement, allowing the temperature to be set inside the container with an accuracy of ±0.1°C. The oscillations of the temperature further facilitates amplification of DNA or cDNA if the proper biochemical conditions are present within the container (47-51, 72, 73).

The device represents a unique and novel combination of methodologies allowing rapid sampling, sample preparation, and DNA or cDNA single molecule amplification integrated in one device.

BRIEF DESCRIPTIONS OF FIGURES

FIG. 1: Cooling of a sample to equilibrium at a 50 °C target temperature. Also shown is the tolerance band at ±0.5 °C of the target temperature, the upper region of which is reached at approximately 4.5 time constants from the start of cooling

FIG. 2: Cooling of a sample to equilibrium at a 50 °C target temperature, magnified view of the region close to adequate equilibrium. As evident, the sample temperature is near the target temperature, but not close enough to be within the tolerance band, for an appreciable time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The term "Fluid" as used herein refers to any fluid, including air, a gas, or a liquid, including water and an aqueous solution.

Embodiment 1.

The design embodies a container or reaction chamber that has dimensions being (or scaling proportionally to) 4.5 mm × 1 mm × 300 μm. The proposed device structure follows the essential schematics of the described concept design.

A silicon substrate base is coated with a thermally resistive layer to define the thermal properties of the device. Subsequently, a resistive heater layer with low resistance, lead out tracks, and contact pads is deposited and patterned. The heater is patterned uniformly to heat the base of the channel. Next a dielectric layer is deposited to insulate the heater from subsequent layers. Then a platinum film is deposited, and patterned to provide temperature sensors and electrodes. The temperature sensors are then selectively insulated from the sample. The silicon wafer fabrication is completed by the processing of any layers that are required for bonding and sealing in the channel formation / lid attachment.

The lid is made of glass to give inherent thermal insulation. A platinum electrode film is deposited and patterned. If required it may be insulated, or it may have temperature sensors patterned in it. The patterning steps are essentially a subset of those used in the silicon wafer fabrication. Fluid access ports are then formed in the lid, a range of processes is possible—but this could be essentially conventional machining. Fluid interface components, such as Luer fittings are bonded to these ports at a convenient stage in the assembly flow.

The channel is defined either by etching into the lid prior to the platinum electrode formation. This is readily possible only if the electrodes on the lid are of relatively coarse geometry allowing trivial fabrication of patterns. Alternately, the lid remains a planar component and the channel is formed in a 300 μm thick "spacer" layer, which is bonded to both the silicon base and the glass lid.

Process layers step by step

In this sub-section we define the layer sequence used to build the device. We outline the functional role(s) of the layer in the device and we give several alternatives in how to obtain the device and list advantages/disadvantages of the choices.

Step 1, see Figure 3

Silicon substrate. This is a silicon wafer in the order of 500 μm thick. A double sided polished wafer is preferable as it facilitates thermal contact to the rear face of the reaction chamber. If lateral thermal isolation can be achieved by a back surface deep RIE process, then either the wafer thickness should be reasonably closely specified or the etch depth varied to compensate for wafer thickness variations. Such wafers are readily available from a large number of sources.

Step 2, see Figure 4

Thermal insulation layer. This layer is deposited on the upper surface of the silicon in order to control the rate of heat loss to the heat sink, and thereby the power required both to maintain the temperature and to heat the sample. For the required PCR cycle times and power levels a polyimide layer of order 20 μm thickness will be required. Various kinds of polyimide are available for use, with BCB (Benzocyclobutene) being an alternate polymeric dielectric material.

Step 3, see Figure 5

Heater layer. This layer is formed in a conveniently resistive material. In the thermal model peak power, dissipation in the heater of order 4 W is indicated. The resistance that it requires depends on the desired drive voltage and current. Currents in the order of 100 mA to 1 A are considered reasonable, with correspondingly voltages ranging from 40 V to 4 V. The required resistance is then in the range of 400 Ω down to 4 Ω. The heater resistor geometry may conveniently closely follow that of the reaction chamber, being a simple rectangular slab covering the entire base. Thus the resistor is approximately 4.5

squares in length indicating a film resistivity in the approximate range 1–100 Ω/m. This resistivity can readily be achieved with a thin film process. A NiCr resistor can be deposited by sputtering, or evaporation, and patterned by wet etching. NiCr resistor processes meeting the requirement are widely available.

Step 4, see Figure 6

Contact layer 1. This layer is deposited immediately following the NiCr deposition and serves to define contacts to the NiCr resistor. Typically this layer would be a thin film of gold. It is defined by wet etching. Self-evidently the pattern is such that there is always NiCr under the gold, but there may not be gold over the NiCr. The typical sequence is a photolithographic mask to define a pseudo-layer "gold OR NiCr", etch the gold then the NiCr, remask to define the layer "gold" and re-etch the gold. (There seems essentially no attack on the NiCr film by iodine based gold etchants). Suitable processes are widely available.

Step 5, see Figure 7

Electrical insulation 1. This layer serves to insulate electrically the heater layer from subsequent conductor layers. The most likely material to be used is a PECVD oxide. The required via / contact openings can be pattered in the oxide using a standard reactive ion etch process. Both the PECVD and RIE processes are widely available.

Step 6, see Figure 8

Platinum 1. This layer serves to form both meander structures used as temperature sensors and to form electrodes used in the spore trapping and DNA detection processes. It is envisaged that the film will be patterned by lift-off photolithography and deposited by electron beam deposition. Instead it is possible to use sputter deposition and either sputter back-etching or a reactive ion etch. However, the lift-off route is likely to be the more readily available.

Step 7, see Figure 9

Contact 2. A second contact layer material may be required to facilitate wire bonding to the platinum films.

Step 8, see Figure 10

Electrical insulation 2. This layer serves to insulate the temperature sensors from the sample. A PECVD oxide deposited and patterned essentially as step 5 is envisaged.

Step 9, see Figure 11

Planarization 1. If the attachment of the lid requires an especially flat surface then it might be necessary to deposit an overall layer which can be polished to a high degree of flatness, before being removed from everywhere except the lid bonding region. Such planarization processes are increasingly common and are most effective. For example, the liquid crystal on silicon display industry uses processes of this type to allow optical quality mirror surfaces to be formed over dense integrated circuit topography.

Step 10, see Figure 12 and Figure 13

For completeness at this stage we show a "picture frame" spacer and the device lid. These are discussed, below. The lid is assumed to have an overall platinum layer or a titanium–gold layer. The notch cut out in the sidewall of the frame is to allow a contact to be brought from the platinum film on the lid down to the main silicon substrate. This is achieved using an injected conductive material, such as a loaded epoxy.

The final device is shown in Figure 14.

Other process information

Glass cover fabrication

A glass cover is preferred since it offers an effective thermal barrier in a proven material. Slight complexity is introduced by the need to define a platinum structure on this lid, and by the fluid ports, but this is not out of the ordinary.

A key design decision is whether to include any temperature sensing and heating on the cover. If these are included then the process flow is more complicated.

A simple cover having only an exposed platinum electrode used for spore capture could be produced by simply sputtering or electron beam evaporating platinum over the entire glass substrate. If heaters and temperature sensors are incorporated then a process flow selected from steps 3–9 of the base silicon process would be used. There may be little variation in detail of steps of the flow to accommodate the glass as opposed to the silicon substrate.

Once the thin films are fabricated the fluid ports would be machined through the glass. Various means are available for this e.g. using a micro-milling machine with a high speed spindle. Other routes include spark erosion, ultrasonic milling, and sand blasting.

Channel formation and device assembly

The channel is formed using a spacer layer bonded to both the silicon and the glass. Many routes are possible for forming and bonding this layer. Our preferred routes are adhesive films and activated or simply clamped PDMS. The alternatives for channel formation is as follows:

A. Adhesive bonding

At the simplest possible level it may be possible to define the channel by die, or laser, cutting into a 300 μm thick film adhesive. Such adhesives are widely used in the mass-market for medical assay devices. However, there may be concerns over the long-term reliability for the current application. Using adhesive tape would be possible if the device were used as disposable.

PDMS molding and plasma assisted bonding

In this process a gasket is moulded in silicone rubber. Such mouldings are readily produced in moderate numbers, with prototypes being cheaply produced. The moulding process allows the retention of very fine detail. The process of bonding silicone rubber to glass by oxygen plasma activation of the silicone is well established in many groups. It readily forms long lasting bonds having high bond strength: typically the silicone rubber material fails, through tearing, before the bond to the glass fails. Since the silicon fabrication completes with a PECVD oxide layer a PDMS gasket can be used to join the two components and form the seal.

If the package and the device are designed together then it may be attractive to use a clamped seal. This would most likely be PDMS. This might be laser, or die, cut from a sheet precision moulded to a thickness marginally in excess of the desired 300μm. Precision spacers in the assembly would be used to limit the compression of the seal and hence limits lateral excursion in compression.

B. Glass–glass fusion bonding

Fusion bonding of glass components is possible provided that both surfaces are very flat and clean. The major qualification over this route is that the temperatures required may prove to be too high to allow the use any polymeric materials in the device.

C. Cold welding

An interesting possibility is to form a frame defining the channels in thin glass, or perhaps to define the channel by etching into the lid. A thin film metallization is then deposited and pattered to define the sealing area and electroplated with a layer of indium. This layer is of order 25 μm thick, with a similar layer being plated onto the seal region of the other components. To assemble the components the indium is cleaned to remove surface oxide, typically with dilute HCl, and then rinsed with solvents. The two clean indium surfaces are then brought into contact and immediately cold weld together.

Claims:

1. A micro scaled device for collecting biological particles, extracting genetic material, and conducting temperature dependent biochemical reactions comprising;

a reaction chamber having an inlet opening providing an air flow capability between the air to be sampled and the reaction chamber, and an outlet opening providing an air flow capability between the reaction chamber and the exterior of the reaction chamber, the outlet or inlet being connected to an air-flow producing means for drawing the air sample through the reaction chamber from the inlet opening to the outlet opening; said reaction chamber having a capability for introducing biochemically defined solvents into the chamber, a capability for removing the products of the temperature dependent biochemical reaction from the chamber, and a capability for very fast and accurate control of the temperature of the reaction chamber, and a collecting and electrolyzing component arranged within the reaction chamber between the inlet opening and the outlet opening, said collecting and electrolyzing component consisting of two or more electrodes positioned in parallel and having the surfaces or at least a part of the surfaces coated with or consisting of material capable of leading an electrical current.

2. The method of claim 1, wherein said parallel electrodes enable the generation of an electrical field at an angle or perpendicular to the air-flow passing through the device, facilitating particles present in the sampled air to become electrostatically charged and thereby being captured by adhering to either the positively or negatively charged electrode.

3 The method of claim 2, wherein said electrodes apply a high frequency alternating electrolyzing field to said captured biological particles after the introducing said biochemically defined solvents.

4. The method of claim 1, wherein said solvents comprise reagents that enable conducting a polymerase chain reaction or PCR.

5. The method of claim 1, wherein said solvents comprise reagents that enable conducting a ligase chain reaction or LCR.

6. The method of claim 1, wherein said solvents comprise reagents that enable conducting a transcription-based amplification.

7. The method of claim 1, wherein said solvents comprise reagents that enable conducting a restriction-based amplification.

8. The device of claim 1, wherein said reaction chamber is adapted to contain in the range of approximately 0.1 µl to 500 µl of fluid.

9. The device of claim 1, wherein more precisely said reaction chamber is adapted to contain in the range of approximately 1.0 µl to 5 µl of fluid.

10. The device of claim 1 combined with the method of claim 7, wherein said reaction chamber has approximately the dimensions of 4.5 mm × 1 mm × 300 µm or proportionally smaller.

11. The device of claim 1 wherein said device is reusable and is fabricated from the group of materials consisting of polymers, silica, glass, metals, and ceramics.

12. The device of claim 1 wherein said device is disposable and is fabricated from the group of materials consisting of polymers, silica, glass, metals, and ceramics.

13. The device of claim 1, wherein said electrodes comprise at least one plate electrode for leading an electrical current thereby creating an electrostatic field.

14. The device in claim 1, wherein said electrodes comprise at least one linear electrode for leading an electrical current thereby creating an electrostatic field.

15. The method in claims 2 and 3, wherein the distance between the electrodes at an angle or perpendicular to the air-flow through the device is between 0.01 mm and 4 mm.

16 The method in claim 2, wherein the applied electricostatic field between the electrodes is between 100 V/mm and 1600 V/mm

17. The method in claim 3, with lysis induced by the application of high frequency alternating electrical fields.

18. The method of claim 17, more precisely with applied alternating current at frequencies between 8000 and 200,000 Hz.

19. The method of claim 17, more precisely with applied pulse sequences between 1 second and 60 seconds.

20. The device of claim 1, further comprising a means of transmission for reporting the results of said biochemical reaction

21. The device of claim 20 wherein said means of transmission is by wire connection, by radio link, by infrared transmission, by microwave transmission, by cellular phone, by GSM module, or by computer network.

22. A microbial monitoring system according to claims 20 and 21, wherein said microbial monitoring system comprises a network of separate devices.

23 A microbiological monitoring system according to claim 22, wherein said monitoring network is an integrated network.

24. The microbial monitoring system of claim 22, wherein said location is determined by means of a global positioning system.

25. The methods of claims 1 - 7, wherein said methods constitute a detection or diagnostics assay.

26. The method of claim 25, wherein said detection assay comprises a variable or programmable timer determining the frequency of detection assays.

FIGURES

FIGURE 1

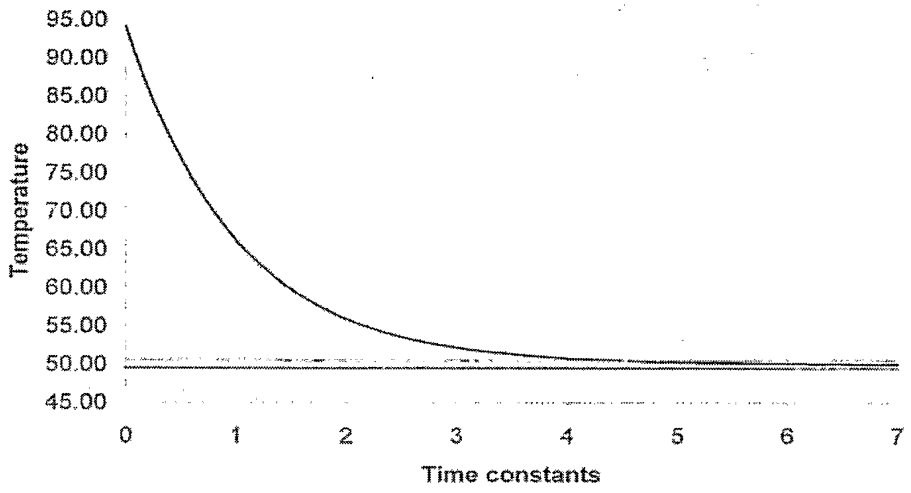

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FIGURE 2

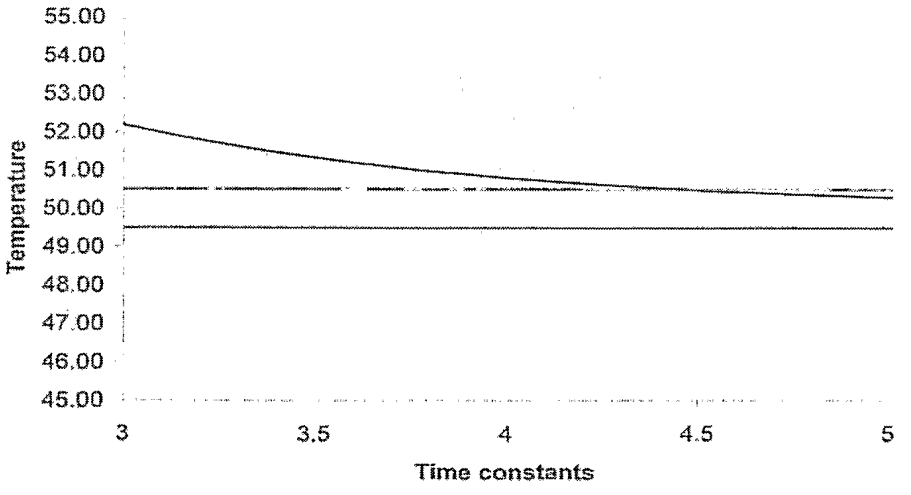


FIGURE 3

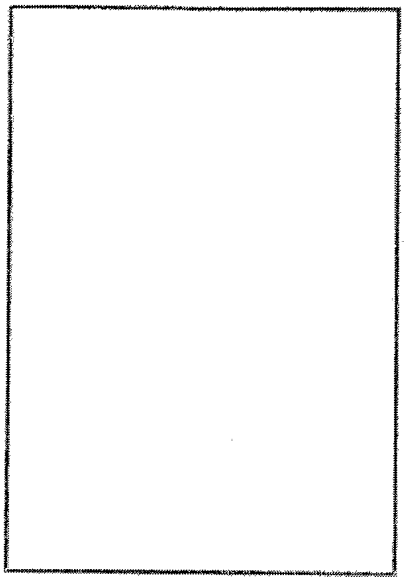


FIGURE 4

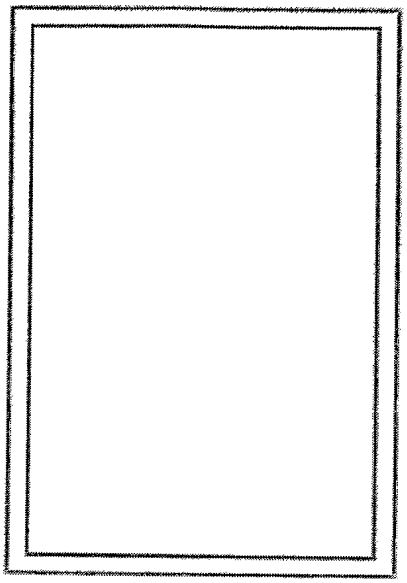


FIGURE 5

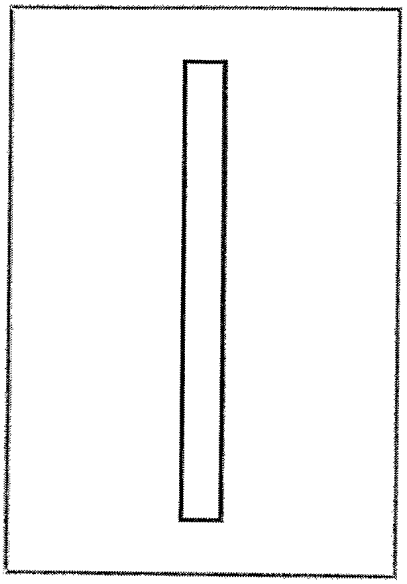


FIGURE 6

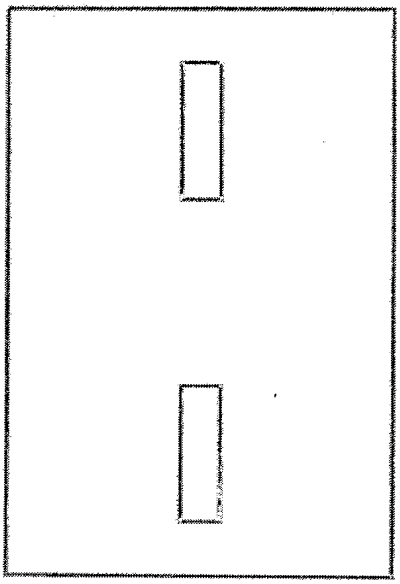


FIGURE 7

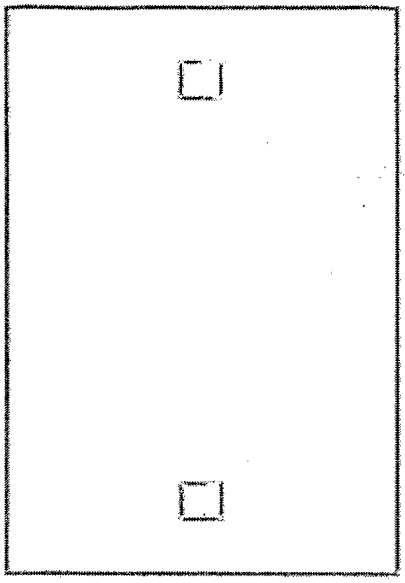


FIGURE 8

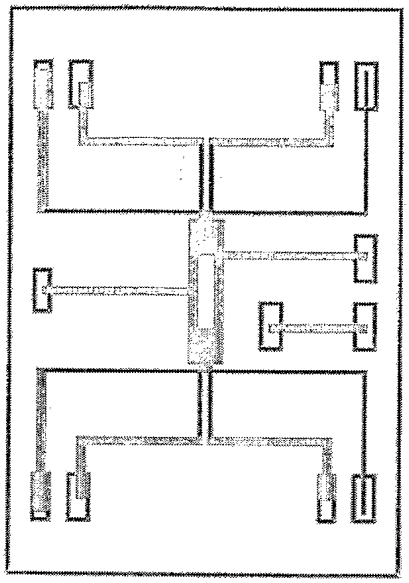


FIGURE 9

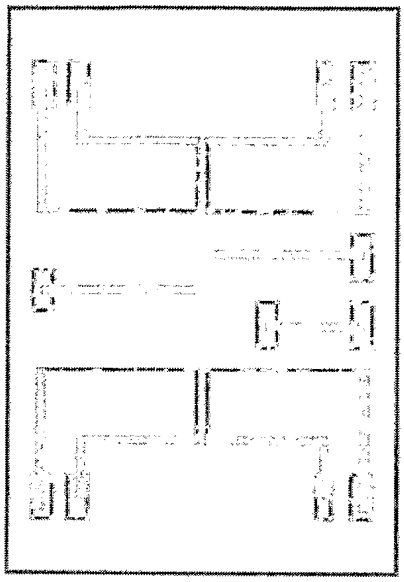


FIGURE 10

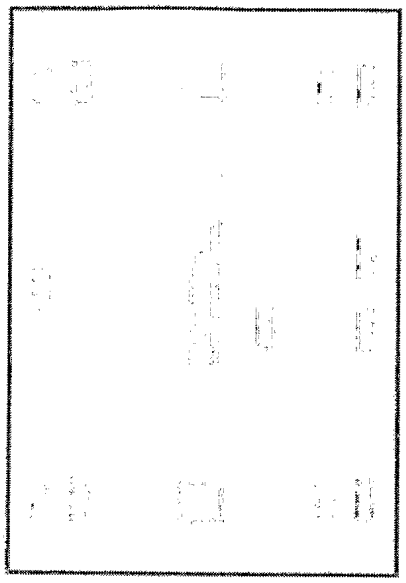


FIGURE 11

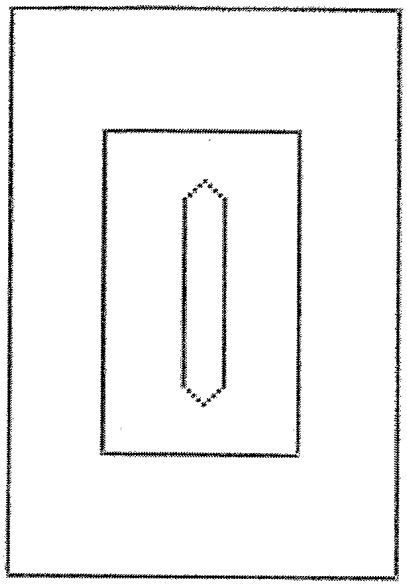


FIGURE 12

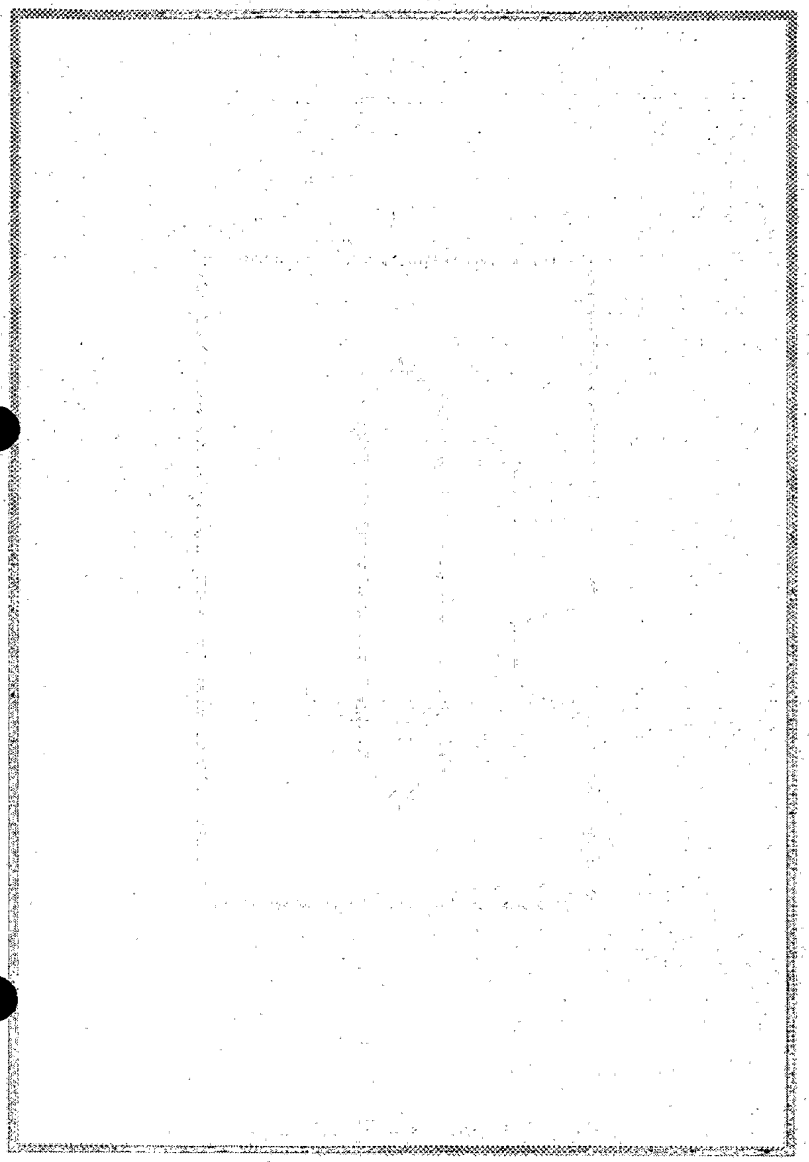


FIGURE 13

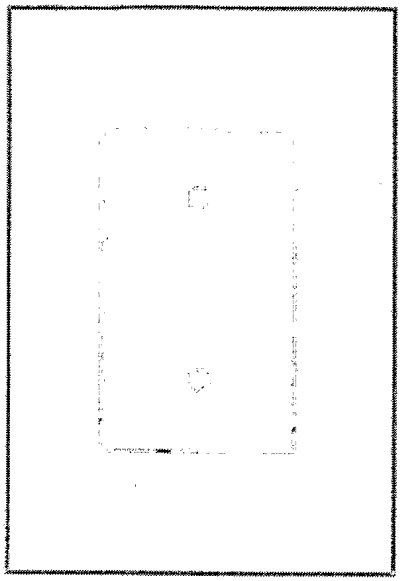
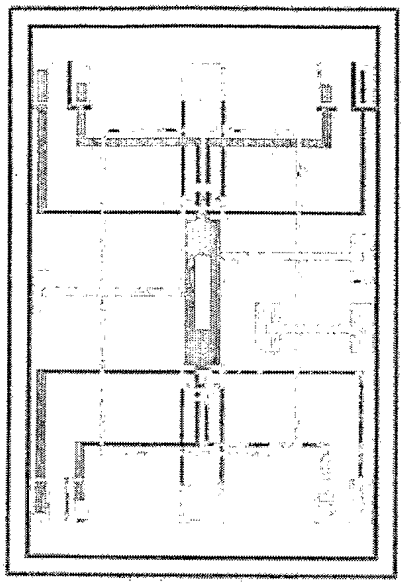


FIGURE 14



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